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14. ABSTRACT

The first year of this grant has already resulted in an informative set of DNA, high risk pedigrees, and phenotype data for a set of pedigrees representing an excess of a highly significant clinic subset of prostate cancer cases: those who will go on to die of the disease. Our initial linkage analysis of less than 200 cases has already significantly confirmed a previously reported linkage on chromosome 1. In addition, our initial linkage analysis of less than 200 cases has already identified a significant linkage on chromosome 11. This initial linkage manuscript is in preparation. We will now collaborate (with other funding) to begin sequence analysis of the regions of interest. Identification of genes predisposing to recurrent/lethal prostate cancer from this study will validate this powerful approach, which can be extended to other high risk prostate cancer pedigrees, and will identify genes and pathways that can be further examined to expand our knowledge of prostate cancer genetics.

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Introduction

Decades of investigation into the genetic causes of prostate cancer and prostate cancer aggressiveness has yet to clearly identify genes or variants which explain much more than a small amount of risk for prostate cancer among a small population of men. Even less progress has been made in understanding why 30% of all patients with localized prostate cancer eventually develop recurrent, and subsequently fatal, prostate cancer, or in understanding the factors that are associated with the range of treatment response (survival time) after diagnosis and treatment for recurrent prostate cancer.

Here we propose a genetic study of the distinct subset of recurrent prostate cancer cases: those who will, in all likelihood, go on to die from their prostate cancer. We will further stratify and study these cases by their response to castration (chemical or physical) treatment, the standard of care for patients with recurrent prostate cancer. Using a unique and powerful statewide, population-based resource, we will identify and sample over 800 recurrent cases of prostate cancer. We will perform genome wide genotyping on informative cases in high-risk pedigrees, and we will apply complementary genetic analyses to identify genes and variants predisposing to recurrent prostate cancer, and variable response to treatment.

Body

Task 1. Recruitment and sampling of new and returning recurrent prostate cancer patients at HCI

We have recruited and sampled 162 new recurrent prostate cancer cases attending Dr. Agarwal's clinic at HCI; with an additional 25 who have agreed to sampling. A DNA sample and prostate cancer questionnaire has been collected and stored for each. We have also identified 27 high-risk prostate cancer kindreds with an excess of lethal prostate cancer and have gathered 196 DNA samples for these historical cases in pedigrees.

sub task 1a. Assignment of phenotype for treatment response for recruited cases

We have recruited a medical student who works with Dr. Agarwal and who has been approved by the Institutional Review Board for this project. He will review charts and enter phenotypes as seen in the Appendix for each case recruited.

Task 2. Identification of most informative samples for genotyping

We have identified 27 high-risk informative recurrent prostate cancer pedigrees with at least 3 samples available for genotyping. We have genotyped 192 (96 x 2) prostate cancer cases in these high-risk informative recurrent prostate cancer pedigrees. These represent prostate cancer cases with survival from 5 months to 232 months after diagnosis of prostate cancer who died with prostate cancer contributing to their death.

Task 3. Genotype 200 samples each year

We have sent 192 (2 plates x 96 samples/plate) samples for genome wide genotyping this year from the high-risk lethal/recurrent prostate cancer pedigrees discussed for Task 2. We have performed genome wide genotyping at the University of Utah Genotyping Core Facility using the Illumina 720,000 SNP Omni Express set of markers.

Task 4. Import and quality control of genotype data

We have acquired genotype data for 192 samples. All data has undergone standard quality control and has been imported into our family study database and linked to phenotype data.

Task 5. Association analysis of all genotyped samples

subtask 5.1 Selection of genotypically matched controls

Using existing software we have analyzed available iControl public data from Illumina for all Caucasians and selected an appropriate genotypically matched set of controls for Utah prostate cancer cases (see Methods in Teerlink et al., 2011).

subtask 5.2 Association Analysis

Because we have not completed genotyping of recurrent prostate cancer cases, and because association analysis requires large sample sizes, we have not begun to analyze the data for associations. We will use existing public software that allows correction for relatedness of cases (GEMMA) when we have additional genotype data in year 2.

subtask 5.3 Validation

We will collaborate with the International Consortium for Prostate Cancer Genetics and other consortia which are also performing association analysis for aggressive prostate cancer to perform a validation of our findings after analysis in year 2.

Task 6. Linkage analysis of all genotyped samples from informative pedigrees

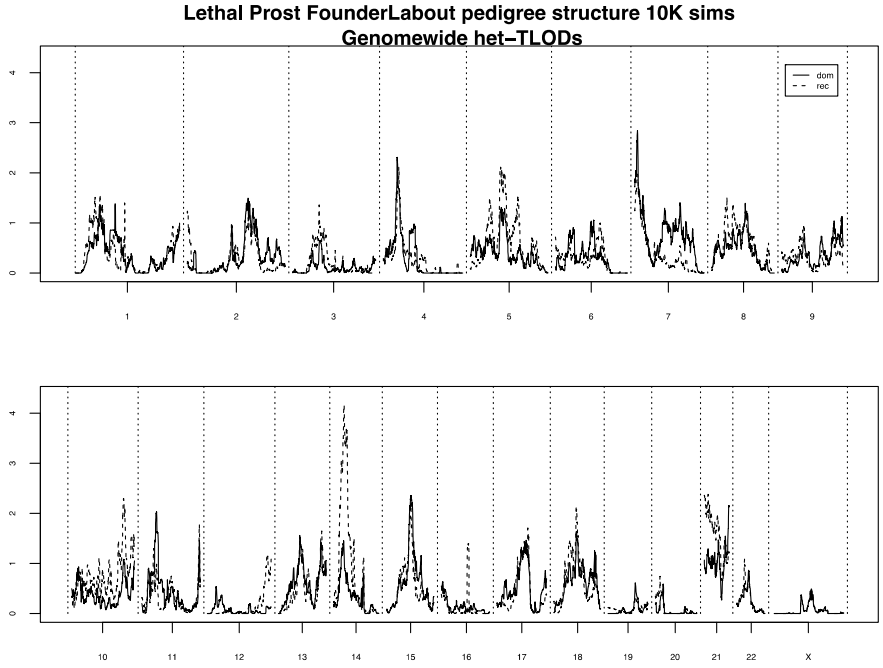
subtask 6.1 Selection of markers for linkage

A set of markers with no linkage disequilibrium must be selected for linkage analysis. We took the intersection of SNP markers from the five Illumina

genotyping platforms that we have used (550K, 610K, 1M,Omni_express (720k), and Omni_1M). This resulted in 301,646 markers. We used an algorithm that began with the first marker on each chromosome and then skipped markers until one satisfied all criteria (min 0.1 cM distance, heterozygosity .35, R-squared < .16 -using HapMap's LD files based on CEPH). This resulted in selection of a set of 25,436 SNP genome wide markers for linkage. See Cannon-Albright et al., 2012 for Methods.

subtask 6.2 Linkage analysis

We have performed genome-wide linkage analysis for 21 of the 27 already genotyped high risk lethal prostate cancer pedigrees with the 25,436 selected SNP markers. Genome wide hetLODs showed one significant linkage peak for the recessive model on chromosome 14 (LOD = 4.20) and are shown in Figure 1. The pedigree with the highest LOD in this region has a +2.69; we are investigating other pedigrees with linkage evidence in this region.



In addition, one pedigree has already provided significant evidence for linkage (LOD > 3.30) for the dominant mode of inheritance. This pedigree has 4 lethal prostate cancer cases and 10 additional prostate cancer cases genotyped. The segregating chromosome 11 haplotype providing linkage evidence is shown in Figure 2.

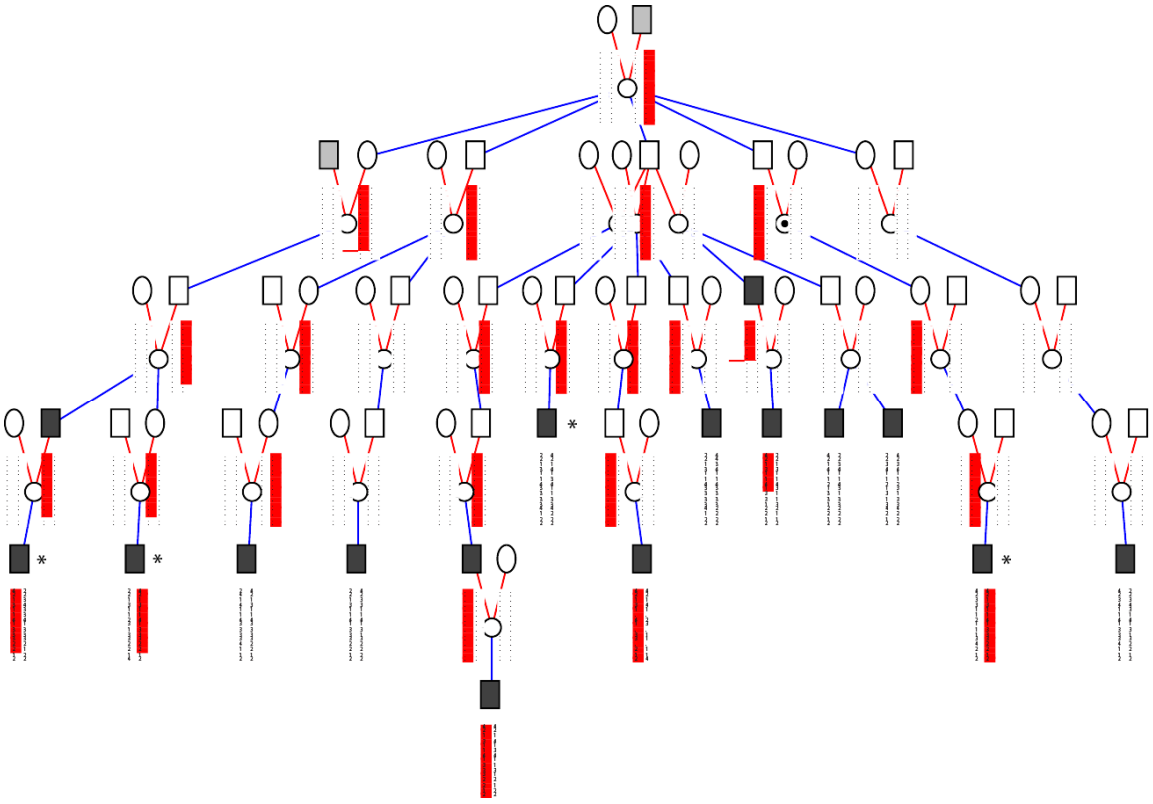


Figure 2. Prostate cancer pedigree with significant evidence for linkage to chromosome 11 (LOD = +3.56). Lethal prostate cancer cases are marked with *. Affected males are fully shaded. The red (dark) haplotype on chromosome 11 that segregates with prostate cancer is shown.

The 1-LOD drop defined support interval for the pedigree linked to chromosome 11 occurs approximately 34-46 cM. This region contains 13 currently documented genomic features, several of which may be reasonable candidates for lethal prostate cancer. The gene PRMT3 (arginine methyltransferase 3) is involved in methylation and is therefore critical to cellular regulation. The protein coded by this gene has demonstrated interaction with the protein product of a tumor suppressor gene DAL-1 (Jiang and Newsham, 2006). The gene NELL1 (Homo sapiens NEL-like 1) may be involved in cell growth regulation and differentiation by influencing the susceptibility of certain promoter regions to hypermethylation (Rappa, et al., 2012; Mori, et al., 2006). The gene FANCF (Fanconi anemia, complementation group F), is a member of a family of genes generally contributing to defective DNA damage repair through the interactions of their protein products (Kusayanagi, et al., 2012). Finally, the gene GAS2 (growth arrest-specific 2) can modulate cell susceptibility to p53-dependent apoptosis (Petroulakis, et al., 2009).

We also observed evidence for dominant linkage at a chromosome 1 locus (LOD > 3.0) for which we have previously published linkage evidence (Camp et al., 2005), this time in a different pedigree which may be related or partially overlap. We are currently sequencing the initial pedigree with significant linkage reported previously, and this new evidence adds linkage evidence to the same region, increasing the chances that we will identify the predisposition gene localized.

Multiple additional pedigrees have provided suggestive evidence for linkage (LOD > 1.90) at several other chromosomal loci, as seen below, including suggestive recessive evidence for linkage (LOD = +3.05) at a different chromosome 1 locus than we previously reported. We will continue to analyze all of these regions and pedigrees, and try to identify additional cases to expand evidence for linkage and to find additional linked pedigrees. Regions that attain significant evidence for linkage will be followed with sequence of predisposition haplotype sharers.

We have only analyzed 21 of the 27 genotyped pedigrees to date; and we will genotype additional pedigrees in Year 2.

Linkage Analysis Evidence for single pedigrees by chromosome

chromosome	**pedigree**	**LOD**	
dominant model:			
chr 1	*324628*	*2.90*	
chr 2	1161095	2.59	
chr 8	337811	2.23	
chr 11	2962217	2.55	(different position)
	352110	*3.56*	**
chr 13	761486	2.29	
chr 15	349943	1.98	
chr 18	345577	2.47	
	355949	2.09	
chr 20	9809999	2.08	
recessive model:			
chr 1	2957225	3.05	(different position than dominant linkage)
chr 5	9809992	2.04	
chr 8	348663	2.67	
chr 14	337811	2.69	
chr 16	9812463	2.17	

subtask 6.3 Validation

Most prostate cancer linkage studies do not use the recurrent/lethal phenotype that we use so these findings may be difficult to validate. We will continue to review

all prostate cancer linkage reports and contact appropriate groups to attempt to validate our regions of interest in high risk prostate cancer pedigrees.

Task 7. Publication of linkage and association manuscripts

A publication reporting the significant linkage finding on chromosome 11 is already in preparation. Analysis of the chromosome 1 new linked pedigree is underway and may lead to a publication.

Key Research Accomplishments

- creation of a set of high risk prostate cancer pedigrees with DNA samples representing an excess of the most clinically significant subset of prostate cases: those with recurrent/lethal disease

- initial linkage analysis of less than 200 cases has already identified a new significant linkage on chromosome 11

Reportable outcomes

- initial linkage manuscript is in preparation

Conclusions

The first year of this grant has already resulted in an informative set of DNA, high risk pedigrees, and phenotype data for a set of pedigrees representing an excess of a highly significant clinic subset of prostate cancer cases: those who will go on to die of the disease.

We have already identified significant evidence for linkage and will now collaborate (with other funding) to begin sequence analysis of the regions of interest.

Identification of genes predisposing to recurrent/lethal prostate cancer from this study will validate this powerful approach, which can be extended to other high-risk prostate cancer pedigrees, and will identify genes and pathways that can be further examined to expand our knowledge of prostate cancer genetics.

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Appendix - Patient Variables

Patient variables
Age at diagnosis
Race
Family History of CaP
BMI
Comorbidities
Concomitant medications
Current Smoking
Prior Smoking
Pack years of Smoking
Pretreatment PSA (one closest to but before initial treatment)
Pretreatment PSA velocity
Clinical Gleason score
Tertiary Gleason Pattern
Clinical Stage (DRE)
Number of cores involved
Highest percent of core involved
Presence LVI
Prostate MRI performed (yes/no)
MRI of prostate findings suggestive of ECE
Pathologic stage (if radical prostatectomy)
Pathologic Gleason Score (if radical prostatectomy)
Pathologic Tertiary Gleason Score
Seminal vesicle involvement
Extra prostatic extension
Lymph node involvement
Number nodes removed
Metastatic disease present at diagnosis
Type of Radiation
Dose of Radiation
Post surgery/radiation PSA
Time to PSA relapse
Post treatment PSA velocity
Date of initiation of ADT
PSA response to castration
PSA response to bicalutamide
Time to imaging progression
Time to initiation of sipuleucel-T
Time to onset of symptomatic metastatic disease
Time to initiation of docetaxel
Best response to docetaxel
TTP on docetaxel
Time to initiation of abiraterone
Best response to abiraterone

TTP on abiraterone
Time to initiation of cabazitaxel
Best response to cabazitaxel
TTP on cabazitaxel
Time to initiation of MDV 3100
Best response to MDV3100
TTP on MDV3100
Concomitant bisphosphonates
Concomitant RANKL inhibitor
Pathologic fractures
Bone Density (Baseline and Followup)
Overall survival